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## ORIGINAL ARTICLE

# Finish line plant–insect interactions mediated by insect feeding mode and plant interference: a case study of *Brassica* interactions with diamondback moth and turnip aphid

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**Abstract** There are gaps in our understanding of plant responses under different insect phytophagy modes and their subsequent effects on the insect herbivores' performance at late season. Here we compared different types of insect feeding by an aphid, *Lipaphis erysimi*, and a lepidopteran, *Plutella xylostella*, and how this affected defensive metabolites in leaves of 2 *Brassica* species when plants gain maturity. Thiocyanate concentrations after *P. xylostella* and *L. erysimi* feeding activities were the same. Total phenolics was higher after the phloem feeder feeding than the folivore activity. The plants compensatory responses (i.e., tolerance) to *L. erysimi* feeding was significantly higher than the responses to *P. xylostella*. This study showed that *L. erysimi* had higher carbon than *P. xylostella* whereas nitrogen in *P. xylostella* was 1.42 times that in *L. erysimi*. Population size of the phloem feeder was not affected by plant species or insect coexistence. However, there was no correlation between plant defensive metabolites and both insects' population size and biomass. This suggests that plant root biomass and tolerance index after different insect herbivory modes are not necessarily unidirectional. Importantly, the interaction between the folivore and the phloem feeder insects is asymmetric and the phloem feeder might be a trickier problem for plants than the folivore. Moreover, as both plants' common and special defenses decreased under interspecific interference, we suggest that specialist insect herbivores can be more challenged in ecosystems in which plants are not involved in interspecific interference.

**Key words** glucosinolates; herbivory mode; plant resistance; stoichiometric analysis; tolerance

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## Introduction

Plants respond to herbivory by chemical changes that result in alterations in patterns of resource allocation to different chemical compounds (Zangerl, 2003; Zhu-Salzman *et al.*, 2005). These compounds are usually carbon (C)-based or nitrogen (N)-based defensive compounds which also vary with season, soil properties, plant age and the

herbivores' feeding damage (Gols *et al.*, 2008a). Plant organs with high turnover rates such as leaves generally contain higher N concentrations than the relatively dormant phloem saps and this N content influences insects that feed on it (Mattson, 1980; Karley *et al.*, 2002; Soufbaf *et al.*, 2012). Tolerance of a plant to herbivory is a genetic trait and is defined as a compensatory response by which a plant decreases the influences of the herbivores attack (Kessler & Baldwin, 2002; Fornoni, 2011). Studies show that plant N has different effects on different herbivores, but fewer studies document induced plant responses to herbivores with different feeding modes with regard to tolerance (guest specific tolerance).

Different herbivory modes have different effects on the response of plants and their consequent fitness. For example, cotton usually responds in a different way to aphid damage than to mechanical damage (Strauss & Agrawal, 1999). Besides, insect herbivores differ in their feeding effects on plant nutrients; for instance, phloem feeders, which imbibe sap from leaves and stems have different effects on plant tissues than folivores that chew and can therefore remove significant amounts of leaf tissues (Throop *et al.*, 2004). On the other hand, studies show that plant responses to different biotic (e.g., herbivory and plant interference) and abiotic (e.g., weather damage and mechanical removal) stresses are often similar and therefore regrowth after damage is likely a generalized plant response to many types of tissue injury. Consequently, a generalized plant response to compensate for damage imposed by different herbivory modes is also expected (Fornoni, 2011). As a defense against insect herbivores, Brassicaceous plants (e.g., cabbages and mustards) typically produce N-based defensive secondary metabolites called glucosinolates (Fahey *et al.*, 2001). However, phenolics are plant's C-based defensive compounds and are a common defensive strategy employed by nearly all plant families. The phenolics are usually induced by low nutrient concentrations (e.g., high C:N) (Dudt & Shure, 1994). Many studies with related plants in the Brassicaceae clearly show that herbivory leads to induced increases in secondary metabolites in shoots soon after leaves are damaged (e.g., see Chen, 2008; Gols *et al.*, 2008b; Gols & Harvey, 2009; Hopkins *et al.*, 2009; Mathur *et al.*, 2013). But, amounts of such induced secondary metabolites have not been studied in the respective plants at late season when plants reach maturity after a long-lasting interaction with the respective insect herbivores.

The ability of plants to tolerate or defend against herbivory is also dependent on other biotic and abiotic variables such as intra- and interspecific competition for nutrients and light as well as microclimatic variables such as moisture and temperature (Rosenthal & Kotanen, 1994;

Strauss & Agrawal, 1999). In particular, competition between neighboring plants can be asymmetric if they compete for limited resources such as sunlight or soil nutrients that are heterogeneously distributed (Donald, 1958; Wilson & Tilman, 1991). Less is known, however, about how interspecific interference between plants can affect herbivores' performance from different feeding guilds such as chewing versus phloem feeding insects.

This study compares the fitness and chemistry of 2 species of insect herbivores with different feeding mechanisms (folivore vs. sap feeding) when reared on 2 *Brassica* species (a modern cultivar of *B. napus* [SLM<sub>046</sub>] and a landrace cultivar of *B. juncea*) that are based on different degrees of domestication. Concentrations of thiocyanate (SCN<sup>-</sup>) (as an index for glucosinolates) and total phenolics (TP) were measured as N-based and C-based plant defensive compounds, respectively. When plants gain maturity immediately following the production of siliques, we determined biomass, population size and elemental chemistry of 2 specialist herbivores, the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) and the turnip aphid, *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae), as well as plant shoots and roots characteristics. Although the plant–insect interactions soon after insect damage have been studied frequently by many researchers, the following hypotheses have not been tested at late season. Definitely, we were not exploring the differences between plant responses at early versus late season, but we tried to visualize such patterns again at late season at which no work had been done hitherto. Our hypotheses were: first, 2 closely related plant species will resist differently (focusing on antibiosis and tolerance) under 2 different insect phytophagies (folivory vs. phloem sap feeding). In other words, *P. xylostella* would induce a plant N-based antibiotic response (SCN<sup>-</sup>) more than a C-based one (TP) because the caterpillar feeds directly on the leaves that are richer in N than C. In contrast, the aphid feeds on the sap that is a rich pool of carbon and therefore C-based defensive compounds are expected. Furthermore, we argued that chewing insects will induce plant tolerance more than phloem feeding insects. This is because the former feed directly on the leaves that are the main site of photosynthesis whereas, the aphids feed on the sap that contains fewer sources of nitrogen and plant biomass. Further, in supporting many similar studies, we showed again that both experimental crucifer species may suffer from interspecific interference between plants when the 2 plant species grow together. Indeed, we only considered plant interspecific interference due to its importance in polyculture while through monoculture plants forcibly experience intraspecific interference. Second and depending on our first hypothesis, *P. xylostella* suffers the plants defensive

chemistry more than *L. erysimi* but suffers coexistence with other insect species less than *L. erysimi*; therefore, we expected the insects' interactions to be asymmetric.

## Materials and methods

### Plants and insects

A modern cultivar, *Brassica napus* L. cv. SLM<sub>046</sub> and a landrace cultivar, *Brassica juncea* L. were used in the experiments. Seeds of the test plants were obtained from Seed and Plant Improvement Institute, Karaj, Iran. Experimental soil was sterilized using 30 KGray gamma radiation to ensure the removal of soil microorganisms. Plants of both species were grown in 20 cm depth soil in pots (20 cm × 25 cm) under greenhouse conditions (27 ± 5 °C, 60% ± 10% RH, natural photoperiod). Plants were watered daily and each pot was caged in a transparent plastic cage (40 cm × 40 cm × 50 cm) well aired on 3 sides with gauze cover. Leaves that were shed because of senescence through the experiment were removed from the cages daily. *P. xylostella* and *L. erysimi* were obtained from the field and maintained on cabbage, *B. oleracea* var. *capitata* L. in the laboratory. The stock cultures were maintained for about 2 months in the greenhouse before trials and insect feces were removed weekly from the cages.

### Experiments

A microcosm experiment on the plant–insect interactions was implemented using *B. napus* and *B. juncea*. The experiment was run in the greenhouse and all measurements were done at the end of the growing season (nearly 45 d after releasing insects into the respective transparent plastic cages when plants gain maturity immediately following the production of siliques). The experiment was conducted at the Agricultural, Medical and Industrial Research School (Karaj, Iran) greenhouse. Plant species were grown in monoculture (1 individual plant of each species in each pot) or in interspecific interference through mixed planting of 2 individuals from 2 plant species (2 different plants grown together in 1 pot) under which competition for nutrients was hypothesized to be established. The experimental insects were released in the respective cages when the plants were 4 week old. The study was designed to examine 2 levels of plant species (*B. napus*, and *B. juncea*) and 2 levels of planting set up (single and mixed cultivation) with 4 levels of insect herbivory factor (*P. xylostella*, *L. erysimi*, *P. xylostella* + *L. erysimi*, and no insect). Treatments without insects

and treatments with the phloem feeder insect had 3 replicates while other treatments had 6 replicates and totally 90 replicates were run under greenhouse conditions with completely randomized design. As the experimental insects could not move between plants (in each cage), for the effect of herbivory on mixed planting of the plant species, plant samplings of both *B. napus* and *B. juncea* affected by both insect species were mixed in the same amounts to get dry weights. Plant shoot and root materials were subjected to oven-drying to get constant dry weights at 80 °C before fine grinding to pass through a 1.25 mm diameter mesh sieve and then the nitrogen and carbon contents of these dried ground materials were estimated by the CHN analyzer (vario EL III, Germany, CHNOS elemental). SCN<sup>-</sup> as an index for glucosinolates in leaf tissues was extracted and measured after a colorimetric test after which SCN<sup>-</sup> ions react with iron III in solution to form an intense red colored complex ion. Absorbance of SCN<sup>-</sup> was measured at 480 nm using UV/vis spectrophotometer (GENWAY, 6705, UK). TP content of leaves was measured following a colorimetric test that uses Folin–Ciocalteu (F–C) reagent. Briefly, to every 96-well multiwell plate, 25 μL of the sample (1, 10, and 1000 μg/mL), 125 μL of F–C reagent (10 % v/v in distilled water), and 100 μL 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> was pipetted. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acid, contained in the F–C reagent, were reduced to blue-colored molybdenum and tungsten oxides. After 1.5 h, the absorbance of blue coloration was measured at 765 nm against a blank sample. The measurements were compared to a standard curve of prepared gallic acid solutions (10, 50, 100, 250, 500, and 1000 mg/L) and expressed as microgram of gallic acid equivalents per mL of sample. All measurements were performed in triplicate per experimental replicate.

To estimate the nitrogen and carbon contents of insects, ground dried bodies of each insect species (oven-dried pupae and adults of *P. xylostella* and *L. erysimi*, respectively, at 55 °C for 12 h) were used in the CHN analyzer (above). Insect samples were weighed to the nearest 10<sup>-4</sup> g using a Sartorius electronic balance (Sartorius GMBH, Göttingen, Germany). As the experimental insects (immature stages of both insects that are direct feeders on the respective plants' foliage) could not move between plants, insect samples for chemical analyses had 3 forms: samples taken from *B. napus*, samples taken from the *B. juncea* and samples taken from *B. napus* + *B. juncea* (insect samples from *B. napus* + *B. juncea* were mixed in the same amounts to obtain dry weights).

For both insects, dried mass and population size were determined when reared on the different plant species. For dried mass measuring, weights of 6 cohorts of 3 individual

insects per treatment were recorded. The phloem feeder population size estimation involved placing the above-ground part of plants individually in an oven preheated up to 60 °C for 20 min, estimating the number of apterous and alates and then the sum of apterous and alates in each replicate (after heat extraction method proposed by Raworth *et al.*, 1984). However, for the folivore insect, population size was estimated based on absolute counting of larva, pupa, and adults in each replicate.

### Statistical analyses

All measurements on 2 plant species were subjected to the Kolmogorov–Smirnov test for conformity to a normal distribution before analyses. Data on nitrogen, C : N ratios and both insects' population sizes were log-transformed and data on root biomass were square root-transformed to give a normal distribution. The data on insect chemistry, population size and weights, as well as plant chemistry (elemental and SCN<sup>-</sup> and TP contents) and weights were analyzed using general linear model procedure considering a full factorial model with plant species, planting set up and insect treatment as fixed factors (Proc GLM, SPSS, 2008). Means were compared by the LSD test at  $P < 0.05$  (SPSS, 2008). Correlations between insect herbivores' performance and plants defensive compounds were estimated using nonparametric Spearman's coefficient ( $\rho$ ) flagging significance at  $P = 0.05$ . We measured tolerance index (TI) after Robinson *et al.* (1991): Tolerance index (weight) (TI) =  $100 \times (\text{mean dry weight of control plants} - \text{mean dry weight of infested plants}) / (\text{weight of insects} \times \text{mean dry weight of control plants})$ . Mann–Whitney U test and Kruskal–Wallis test of the TI were performed for the 3 insect populations (pure *P. xylostella*, pure *L. erysimi*, and mixed *P. xylostella* + *L. erysimi*) and 3 plant treatments (2 monocultures and 1 mixed planting).

## Results

### Plant elemental chemistry

Nitrogen and C : N ratio of the experimental plants' foliage were different between different plant species and different planting set ups (Table 1) while plant N was different under different phytophagy modes at 10% significance level ( $F_{3,57} = 2.61$ ,  $P = 0.06$ ). However, there was no significant difference of carbon concentrations between treatments. The highest N was recorded in *B. juncea* in a singular planting set up without insects while the lowest N was recorded on mixed *B. napus* under ac-

**Table 1** Analysis from the carbon, nitrogen and C : N ratio of 2 experimental plant species, *Brassica napus* and *Brassica juncea* under effect of plant species, planting set up, and insect phytophagy mode using a linear model approach.

Source	Dependent variable	Type III sum of squares	df	F
Intercept	Carbon	110273.668	1	21706.23***
	Nitrogen	1.994	1	107.33***
	C : N	133.556	1	6535.97***
Plant species	Nitrogen	0.434	1	23.36***
	C : N	0.427	1	20.89***
Planting	Nitrogen	0.418	1	22.50***
	C : N	0.389	1	19.01***
Plant species × Planting × Insect	Nitrogen	0.239	3	4.28**
	C : N	0.242	3	3.95*
Error	Carbon	289.576	57	
	Nitrogen	1.059	57	
	C : N	1.165	57	
Total	Carbon	124603.101	73	
	Nitrogen	4.336	73	
	C : N	157.073	73	

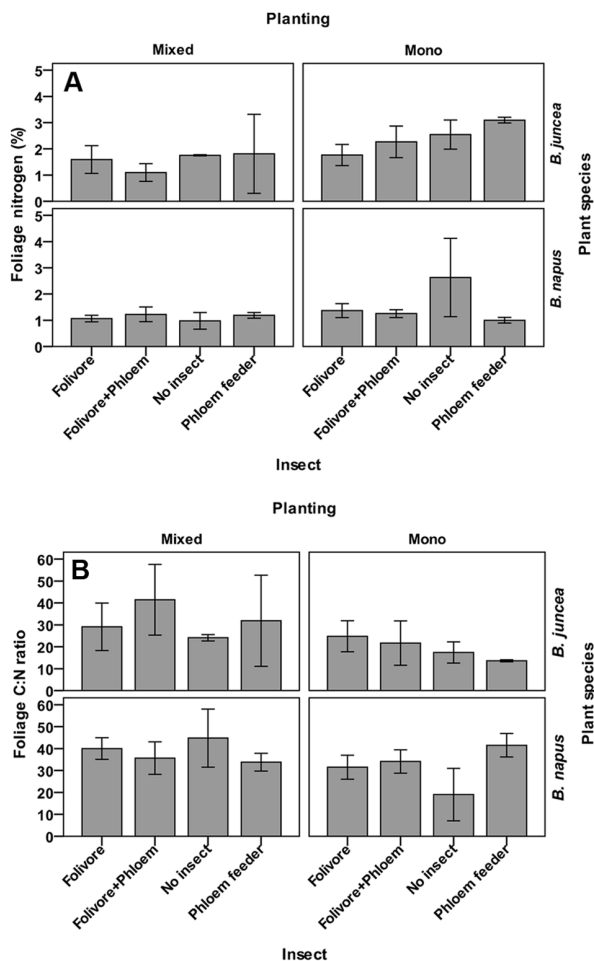
Note: The only significant main effects and their interactions were shown here.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

tion of either folivore phloem feeder or combination of both insect species (Fig. 1A). The lowest C : N ratio was observed on *B. juncea* in a single planting set up without insects and its highest value was obtained on mixed *B. napus* under action of either folivore, phloem feeder or combination of both insect species (Fig. 1B).

### Plant secondary metabolites

SCN<sup>-</sup> was different between different plant species, planting set ups and insect treatments (Table 2) and TP of the experimental plants varied among different insect treatments as well as different planting set ups (Table 3). The presence of herbivores (either *P. xylostella*, *L. erysimi* or both) caused decreased SCN<sup>-</sup> and TP (Figs. 2A, B). The highest SCN<sup>-</sup> was measured on *B. juncea* in single planting; however, the lowest SCN<sup>-</sup> was measured on the mixed *B. napus*. Also, the lowest SCN<sup>-</sup> was recorded in plants with either *L. erysimi* or *P. xylostella* (either singly or with each other). In other words, the highest SCN<sup>-</sup> was observed when there were no insects of either species on the singular cultivated *B. juncea* (Fig. 2A). The highest and lowest TP was obtained in single and mixed planting, respectively (Fig. 2B). However, the effect of phytophagy



**Fig. 1** Mean  $\pm$  SE of nitrogen percentage (A) and C : N ratio (B) of *Brassica napus* and the *Brassica juncea* under 2 different planting set ups (single vs. mixed planting) and 4 insect treatments at late season.

modes on the TP was significant such that the pure phloem feeder and pure folivore caused the highest and the lowest TP, respectively. Also, TP under the action of both insect species together was higher than when the folivore fed singly and lower than when the phloem feeder acted alone (Fig. 2B).

#### Plant tolerance

There was a significant difference of the tolerance index (TI) between the plants exposed to 3 insect phytophagy modes (only folivore, only phloem feeder, folivore + phloem feeder) ( $P = 0.002$ ) and 2 planting set ups ( $P = 0.005$ ) while the plant species show a similar tolerance response ( $P = 0.62$ ) (Fig. 3). There were, however, significant differences of nitrogen and C : N values of

**Table 2** Analysis from the thiocyanate ( $\text{SCN}^-$ ) of 2 experimental plant species, *Brassica napus* and *Brassica juncea* under effect of plant species, planting set up, and insect phytophagy mode using a linear model approach.

Source	Type III sum of squares	df	F
Intercept	349.01	1	2062.03***
Plant species	25.06	1	148.07***
Planting	26.73	1	157.93***
Insect	9.58	3	18.86***
Plant species $\times$ Planting	8.64	1	51.06***
Planting $\times$ Insect	12.71	3	25.03***
Plant species $\times$ Planting $\times$ Insect	3.96	3	7.80***
Error	5.42	32	
Total	441.90	48	

Note: The only significant main effects and their interactions were shown here.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

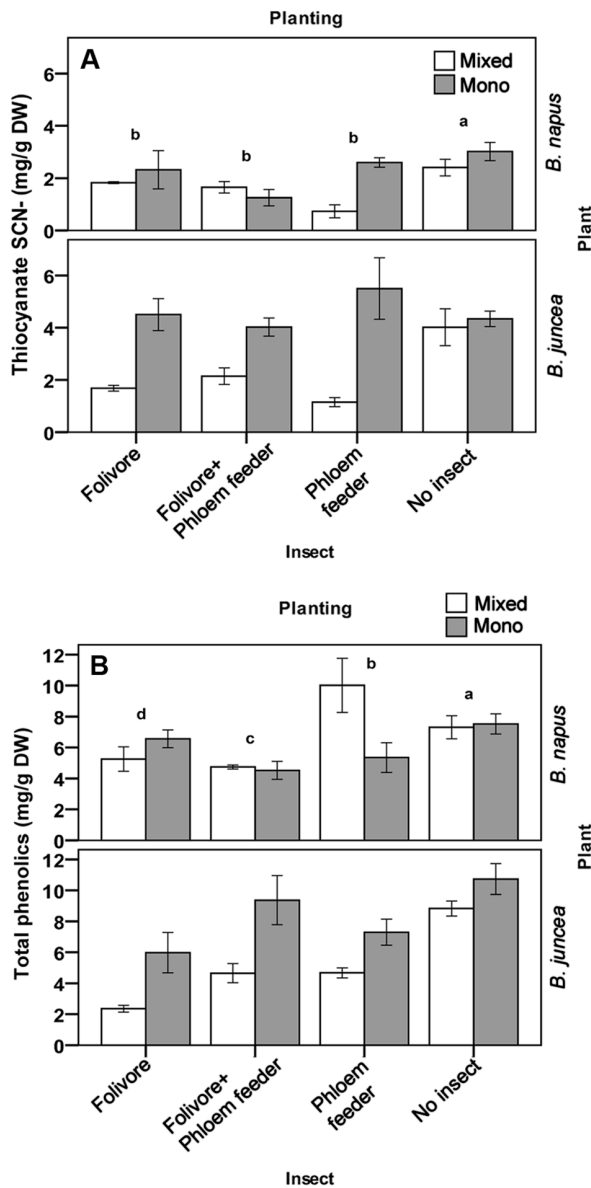
**Table 3** Analysis from the total phenolics (TP) of 2 experimental plant species, *Brassica napus* and *Brassica juncea* under effect of plant species, planting set up, and insect phytophagy mode using a linear model approach.

Source	Type III sum of squares	df	F
Intercept	2072.19	1	3408.22***
Planting	16.96	1	27.91***
Insect	85.12	3	46.67***
Plant species $\times$ Planting	49.45	1	81.34***
Plant species $\times$ Insect	50.21	3	27.52***
Planting $\times$ Insect	22.93	3	12.57***
Plant species $\times$ Planting $\times$ Insect	14.89	3	8.16***
Error	19.46	32	
Total	2332.50	48	

Note: The only significant main effects and their interactions were shown here.

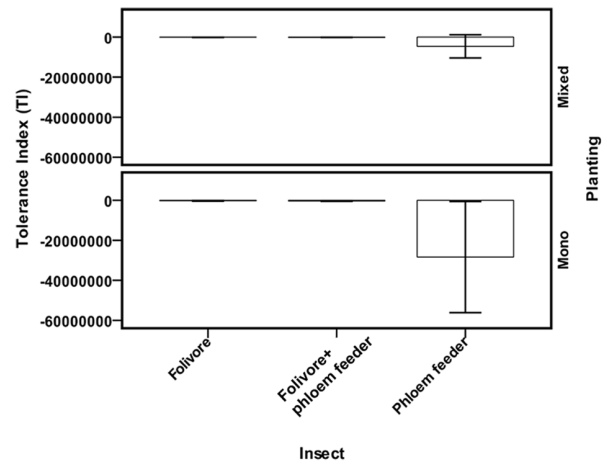
\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

plant roots between different insect treatments, while neither planting set ups nor plant species had a significant effect on the plant root carbon, nitrogen and C : N ratio (see Table S1). The highest nitrogen concentrations in the roots of the experimental plants were obtained when there was no insect feeding on the respective plants (Fig. 4A).

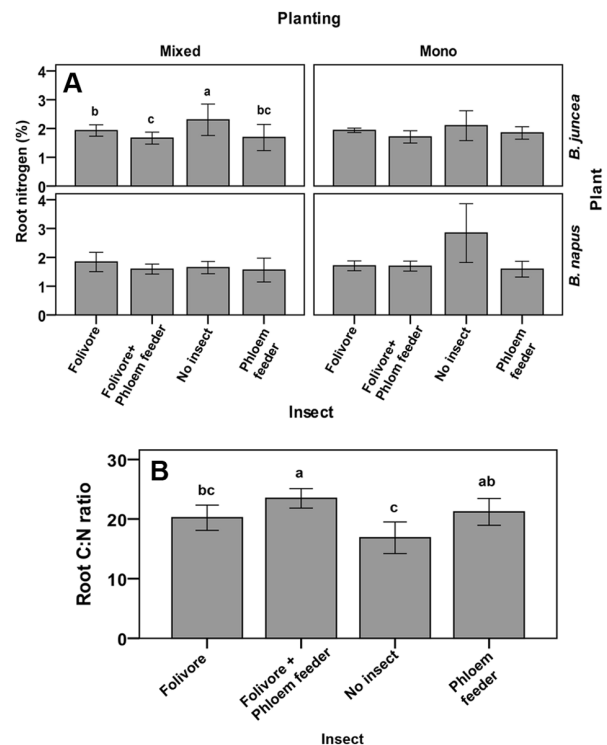


**Fig. 2** Secondary metabolites of *Brassica napus* and *Brassica juncea* under 4 insect phytophagy modes (folivory vs. phloem feeding, in single vs. coexisting scenarios) and 2 different planting set ups (mono vs. mixed planting) under the greenhouse condition at late season. (A) and (B) show thiocyanate (SCN<sup>-</sup>) and total phenolics (TP) of the experimental plants, respectively. Folivore and phloem feeder insects are *Plutella xylostella*, and *Lipaphis erysimi*, respectively. Letters on each error bar (SE of mean) of SCN<sup>-</sup> and TP represent insect phytophagy mean comparisons after LSD test at  $P < 0.05$ .

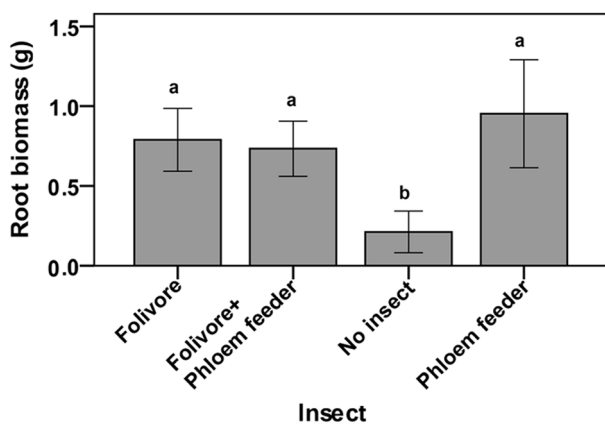
Therefore, the lowest C : N ratio was obtained when there was no insect feeding on the respective plants (Fig. 4B). Root dry weight was not different between 2 experimental plant species and 2 different planting set ups while



**Fig. 3** Tolerance index (TI) of the experimental plant species under 3 insect phytophagy modes (folivory, phloem feeding, and folivory + phloem feeding) and 2 different planting set ups (mono vs. mixed planting) under the greenhouse condition at late season.



**Fig. 4** Nitrogen percentage (A) and C : N ratio (B) of *Brassica napus* and *Brassica juncea* roots under 2 insects phytophagy modes under the greenhouse condition at late season. Folivore and phloem feeder insects are *Plutella xylostella* and *Lipaphis erysimi*, respectively. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.



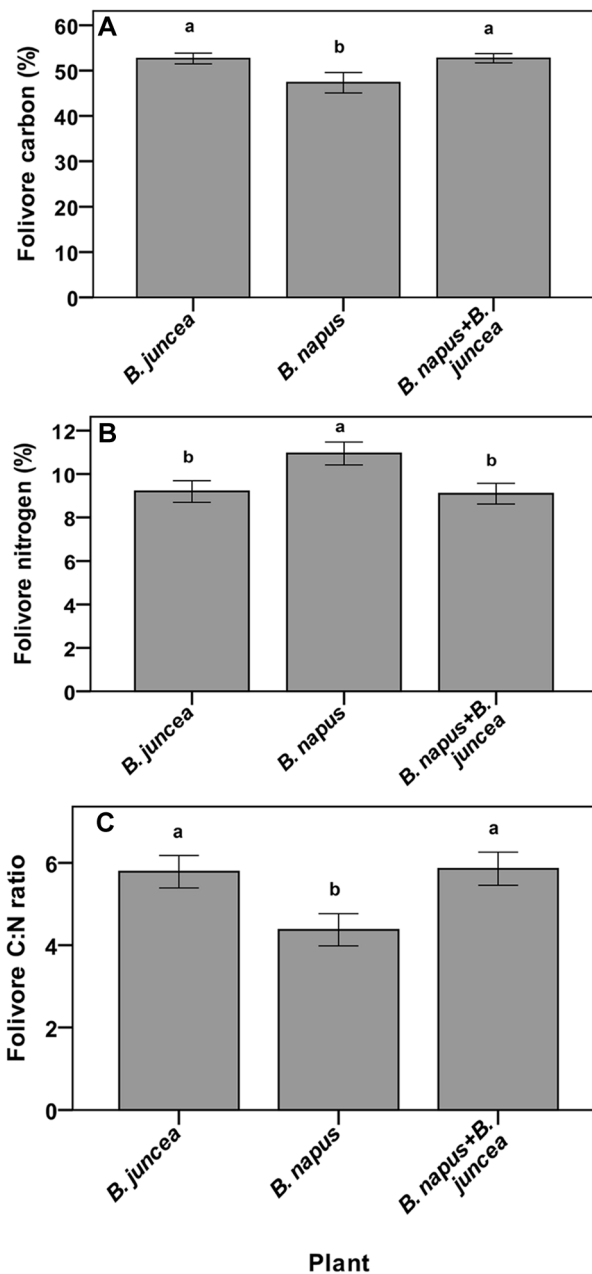
**Fig. 5** Root biomass of *Brassica napus* and *Brassica juncea* under 2 insect phytophagy modes under the greenhouse condition at late season. Folivore and phloem feeder insects are *Plutella xylostella*, and *Lipaphis erysimi*, respectively. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.

insect phytophagy affected the root dry weight ( $F_{1,100} = 0.54$ ,  $P = 0.47$ ;  $F_{1,100} = 2.58$ ,  $P = 0.11$ ;  $F_{3,100} = 6.02$ ,  $P = 0.001$ , respectively). Independent of either herbivore species, insect feeding affected root biomass positively (Fig. 5).

#### Insect herbivore chemistry

***Plutella xylostella*** Carbon, nitrogen and C : N ratio of *P. xylostella* varied among different *Brassica* species assemblages ( $F_{2,30} = 17.21$ ,  $P < 0.0001$ ;  $F_{2,30} = 17.24$ ,  $P < 0.0001$ ;  $F_{2,30} = 17.89$ ,  $P < 0.0001$ ; statistics for C, N, and C : N, respectively) but were not significantly affected by the presence of *L. erysimi* ( $F_{1,30} = 2.47$ ,  $P = 0.13$ ;  $F_{1,30} = 2.52$ ,  $P = 0.12$ ;  $F_{1,30} = 1.94$ ,  $P = 0.17$ ; statistics for C, N, and C : N, respectively). The highest carbon in the insect was recorded when it was reared on *B. juncea* (either in plants grown singly or in mixed planting) and the lowest carbon was observed when it was reared on *B. napus* (Fig. 6A). The highest nitrogen of *P. xylostella* was obtained when it was reared on *B. napus* whereas insects reared on *B. juncea* and mixed planting contained the lowest nitrogen (Fig. 6B). Therefore, the largest C : N ratio was observed when the insect was reared on *B. juncea* (in plants grown either singly or in mixed planting) and the smallest C : N ratio was obtained in insects reared on *B. napus* (Fig. 6C).

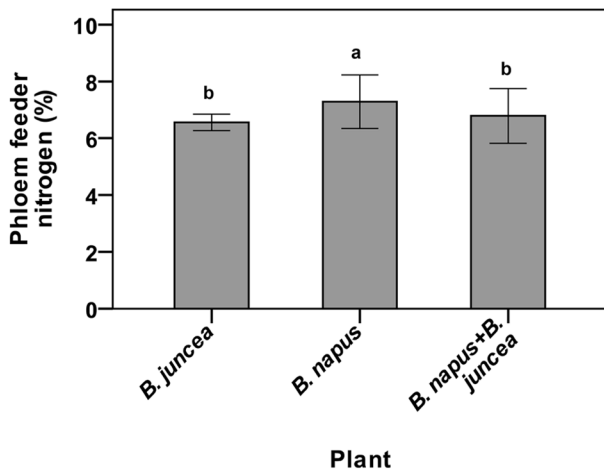
***Lipaphis erysimi*** Nitrogen of *L. erysimi* differed between different plant species assemblages ( $F_{2,21} = 11.27$ ,  $P < 0.0001$ ) (Fig. 7) whereas its carbon and C : N ratio



**Fig. 6** Elemental chemistry of the folivore, *Plutella xylostella* under 3 different planting set ups at late season. (A), (B), and (C) show *P. xylostella*' nitrogen, carbon, and C : N ratio, respectively. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.

were not affected by different plant species assemblages ( $F_{2,21} = 1.12$ ,  $P = 0.34$ ;  $F_{2,21} = 1.46$ ,  $P = 0.25$ , respectively); however, carbon, nitrogen, and C : N ratio of *L. erysimi* were different between 2 insect species ( $F_{1,21} = 31.46$ ,  $P < 0.0001$ ;  $F_{1,21} = 125.5$ ,  $P < 0.0001$ ;  $F_{1,21} = 53.37$ ,  $P < 0.0001$ , respectively). *L. erysimi* contained





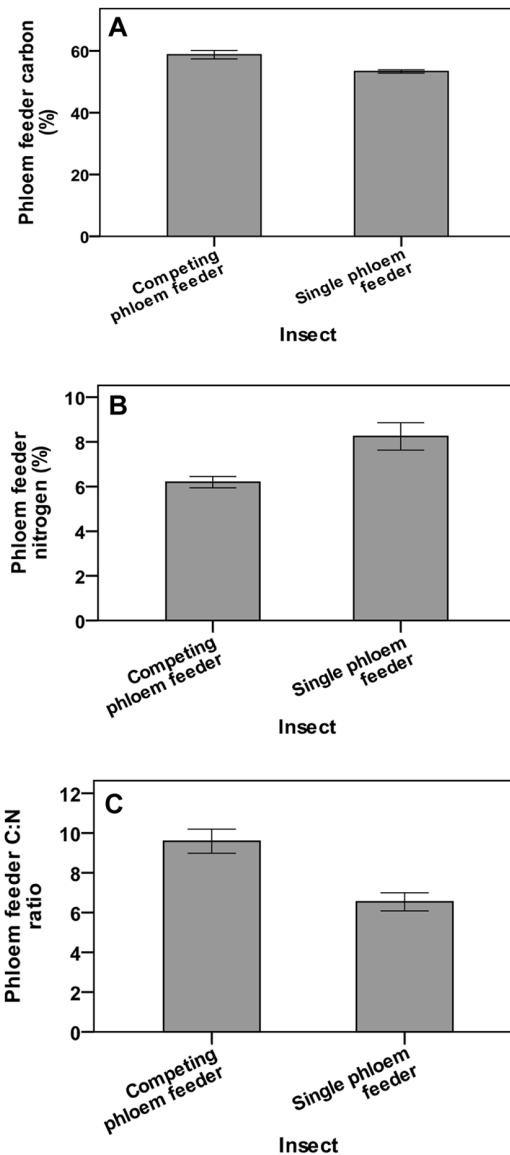
**Fig. 7** Elemental chemistry (nitrogen) of the phloem feeder, *Lipaphis erysimi* under 3 different planting set ups at late season. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.

the highest carbon through competing action of the insect (Fig. 8A) while the insect got the highest nitrogen when reared alone on *B. napus* (Figs. 7 and 8B). Therefore, the largest and smallest C : N ratios were observed through competing and single action of the insect, respectively (Fig. 8C).

#### Insect performance

**Population size** Population size of *P. xylostella* varied among different plant species and set ups ( $F_{2,58} = 13.00$ ,  $P < 0.0001$ ) while the effect of competition with *L. erysimi* on this feature was not significant ( $F_{1,58} = 0.9$ ,  $P = 0.35$ ). The largest population size of *P. xylostella* was found on *B. napus* through single planting and the smallest population size on either *B. juncea* or mixed of both plant species (Fig. 9). Population size of the phloem feeder, *L. erysimi* was not different among different plant species and set ups ( $F_{2,31} = 2.22$ ,  $P = 0.13$ ) or between 2 insect coexistence situations ( $F_{1,31} = 0.31$ ,  $P = 0.58$ ).

**Dry mass** Dry pupal weight of *P. xylostella* differed among individuals reared on different plant species set ups ( $F_{2,47} = 8.47$ ,  $P = 0.001$ ) although competition with *L. erysimi* had no effect on this feature ( $F_{1,47} = 0.03$ ,  $P = 0.86$ ). The highest pupal weight was obtained in insects reared on *B. napus* and *B. juncea* in single planting, whereas the lowest pupal weight was obtained in insects reared under mixed planting (Fig. 10). Further, plant species or competition with *P. xylostella* did not affect

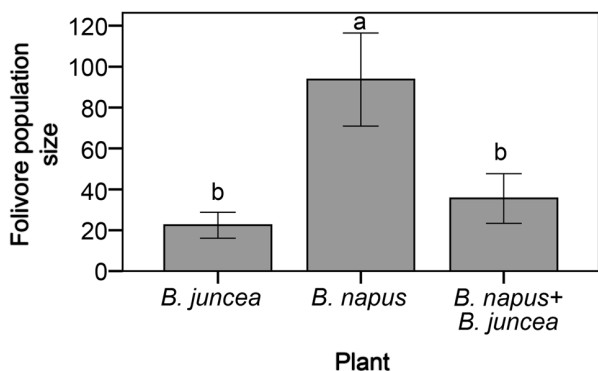


**Fig. 8** Elemental chemistry of the phloem feeder, *Lipaphis erysimi* under 2 different insect actions at late season. (A), (B), and (C) show *L. erysimi* nitrogen, carbon, and C : N ratio, respectively. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.

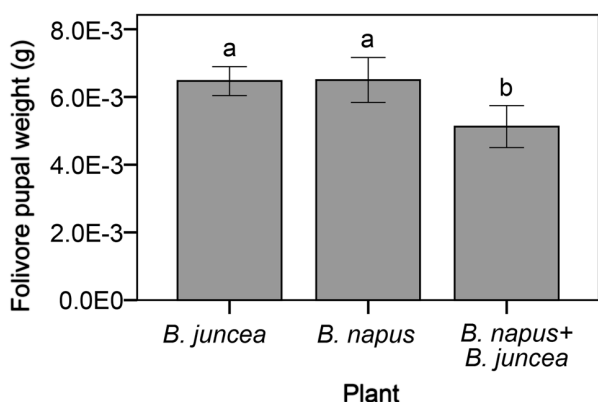
dry weight of *L. erysimi* significantly ( $F_{2,27} = 0.46$ ,  $P = 0.64$ ;  $F_{1,27} = 0.17$ ,  $P = 0.69$ , respectively). There were no correlations between plant secondary metabolites and insect population size or biomass (see Table S2).

#### Discussion

The results showed that in contrast to nitrogen, the N-based defensive compound ( $\text{SCN}^-$ ) increased after insect



**Fig. 9** Population size of the folivore, *Plutella xylostella* under 3 planting set ups at late season. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.



**Fig. 10** Dried pupal weight of the folivore, *Plutella xylostella* under 3 planting set ups under greenhouse condition at late season. In the figure, common letters over the bars are not significantly different at  $P < 0.05$  after LSD test.

feeding activity.  $SCN^-$  concentrations were the same in response to *P. xylostella* and *L. erysimi* damage whereas TP concentrations were higher following damage by *L. erysimi* than *P. xylostella* damage thus only partially supporting our first hypothesis. Importantly, we found higher concentrations of secondary plant metabolites in control plants than after herbivore damage, irrespective about treatment (e.g., feeding alone or together). Defensive compounds were measured after a long-lasting plant–insect coexistence, thus, it could be concluded that increased concentrations of allelochemicals are not maintained after herbivore's feeding but decrease with time. In other words, plants may partially compensate metabolic budgets between early defense following early herbivore attack and other functions (e.g., growth) later in the sea-

son, although the relationship with herbivores has not ended. We assumed that early insects' attack induces high concentrations of defensive metabolites and then the (depressed insect)–(injured plant) 2-sided relationship continues more slightly. Other studies with related plants in the Brassicaceae clearly show that herbivory leads to induced increases in secondary metabolites in shoots soon after the insects injure these tissues (Gols et al., 2008a; Gols & Harvey, 2009; Mathur et al., 2013).

Against the last claim of the first theory, our results revealed that plant compensatory response (i.e., tolerance) to damage by *L. erysimi* was significantly higher than the response to *P. xylostella*. This finding contradicts our expectations and those of other researchers (e.g., Feroni, 2011). This suggests that plants might show genetic correlations in tolerance to different herbivore species (or species in different feeding guilds) and do not show a generalized plant response to different types of damage (see Feroni, 2011). Similarly, some researchers have suggested that tolerance often depends on the identity of the attacking herbivore (e.g., see Gavloski & Lamb, 2000 working on *Brassica napus*, *Sinapsis alba* and 3 insect herbivores). However, when comparing plant tolerance due to 2 very different insect species such that their body size and biomass are very different (like our experimental insect species), then, the denominator of the TI formulae could be a weakness point of comparisons. Importantly, the dry weight of the folivore insect in our experiments was 30–90 times of the phloem feeder insect. Therefore, even considering other terms of the TI formulae the same for both insects, then TI under folivore action would be very lower than the TI value obtained for the phloem feeder. However, considering root biomass as an index for plant tolerance against different insect phytophagy modes fitted to our hypotheses more than TI. Further, the biomass index in this study could be supported completely by the common belief on the general compensatory response of plants to different biotic and abiotic stresses (mapped in Introduction section of this paper).

Concentrations of nitrogen were reduced when the 2 *Brassica* species were grown together, showing the 2 plant species compete for this important and often limiting resource. Nitrogen in the single planting of *B. napus* and *B. juncea* was 1.32 and 1.53 times higher than in mixed plantings with *B. juncea* and *B. napus*, respectively. While TP did not show a consistent response to plant species, both  $SCN^-$  and nitrogen were higher on *B. juncea* and were lower in the mixed planting set up expectedly. Besides, TP was higher under single planting while C did not show a response to planting set up. Overall, elemental chemistry of the experimental plant species was independent of insect phytophagy. Plants that were damaged by

the phloem feeder produced higher amounts of TP than plants damaged by *P. xylostella*. Our results also suggest that both plant species had lower concentrations of secondary metabolites when grown together. However, the C : N ratio was higher in plants grown singly than in mixed plantings. Hodge (2003) showed that both *Brassica napus* and *Plantago lanceolata* uptake less N from organic material when grown together. The harmful effect on N uptake from organic material in mixed set ups was more severe for *P. lanceolata*, which captured only 3% of the total N from organic material whereas 97% were captured by the *B. napus* (Hodge, 2003). This clearly reveals competitive asymmetries between different plant species for access to key nutrients. Moreover, our results revealed the dried weights of plant roots under all experimental conditions were higher when plants were grown alone (1 individual plant). Root dried weight in both *B. napus* and *B. juncea* under single planting was almost 1.7 times greater than in the mixed plating set up in which the plants experienced interspecific interference. However, at agricultural scales in which experimental plants also experience intraspecific interference, researchers showed completely different patterns. For instance, Cardinale *et al.* (2007) showed that mixtures of plant species produce an average of 1.7 times more biomass than species monocultures. Moreover, Cadotte (2013) showed that species produced more biomass than predicted from monocultures when they were in plots with distantly related species and produced for biomass predicted from monoculture when sown with close relatives.

Some of the insect responses to different plant quality and quantity are well documented (e.g., see Low *et al.*, 2009 for the effects of leaf size on a leaf-mining moth and Gols *et al.*, 2008a for the comparative effects of plant domestication on specialist and generalist herbivores). However, few studies have examined insect herbivore elemental chemistry when reared on 2 closely related plant species under competitive coexistence (e.g., see Pankoke *et al.*, 2015 for the effect of *Plantago* defensive compounds on a caterpillar N uptake). This study showed that nitrogen percentage in *P. xylostella* was higher than its value measured in *L. erysimi* across all the treatments. Overall, carbon and C : N ratio in *L. erysimi* were 1.12 and 1.61 times that in *P. xylostella* whereas nitrogen in *P. xylostella* was 1.42 times that in *L. erysimi*. Elemental chemistry of *P. xylostella* was not affected when reared on the same plants with *L. erysimi* in contrast to *L. erysimi* that responded negatively in this feature when reared with *P. xylostella*. It could be concluded that the interaction between these 2 herbivores is asymmetric, thus supporting our second theory well. *L. erysimi* showed the highest carbon, the lowest nitrogen and therefore, the highest

C : N when reared with *P. xylostella*. Nitrogen of both insect herbivores was higher when developing on *B. napus* in the single planting treatment, compared with the other plant treatments. *P. xylostella* herbivory may lead to a decrease in N concentrations in *B. napus* plants. Also, lower  $\text{SCN}^-$  in *B. napus* than *B. juncea* may confirm this argument. *P. xylostella* had the highest carbon when feeding on *B. juncea*; however, the C : N ratio was the highest on *B. juncea* plants that also expressed the highest values of  $\text{SCN}^-$ . In contrast with *P. xylostella*, *L. erysimi* did not show significant differences in carbon when reared among the different plant treatments, which is because this species, like other aphids, feeds on carbon-rich tissues (i.e., phloem sap). Similar to patterns in the nitrogen content of *P. xylostella*, *L. erysimi* also contained higher nitrogen when feeding on *B. napus* even though this plant species had the lowest nitrogen through all plant treatments. Our results revealed that *P. xylostella* was more efficient in getting and storing nitrogen than *L. erysimi* in the different plant treatments. Karley *et al.* (2002) showed that poor aphid performance on tuber-filling plants might have been attributed to raised leaf C : N ratios, which are often considered as a sign of low plant nutritional quality. They suggested that plant indices based on the elemental composition of total plant tissues are not necessarily an accurate index of the nutritional quality of the plant for phloem-feeding insects.

Population sizes of both insect herbivores were not affected by the presence or absence of the other herbivore species. Also, the population size of *L. erysimi* was not affected by the plant species on which it was reared. Interestingly, the presence of *L. erysimi* had no effect on all performance features of *P. xylostella*. Similar to our results, Fisher *et al.* (2000) working on the direct and indirect competitive effects of foliage feeding guilds on the birch leaf-miner *Eriocrania* found that although the presence of aphids reduced the quantity of foliage available by 33%, the development of *Eriocrania* was unaffected. Some studies have suggested that there are physiological mechanisms by which insect growth rates and sizes respond to changes in foliar composition, including increased N-based or decreased C-based secondary metabolites (Mattson, 1980; Throop *et al.*, 2004). A direct influence of plant quality on population size and dynamics is notoriously difficult to prove because of the multiplicity of indirect biotic or abiotic effects that may explain correlations (Hunter & Price, 1998; Clissold *et al.*, 2006). Population size of *P. xylostella* was the smallest on the *B. juncea* that might be related to the highest  $\text{SCN}^-$  found in this plant species. However, *P. xylostella* had the biggest population size on *B. napus* plants that had the highest C : N ratio; however, it must be noted there was no

correlation between plant  $\text{SCN}^-$  and TP on insect population size as well as biomass.

*P.xylostella* pupal dry mass was the highest when reared on single cultured either *B. napus* or *B. juncea* even though under this planting set up, plants showed the highest secondary metabolites. However, highest nitrogen and the lowest C : N ratio of the experimental plants under this planting set up support the last observation on the pupal weight. Also, *B. napus* had lower  $\text{SCN}^-$  than the *B. juncea*, which supports the last finding partially. However, some researchers showed that *P. xylostella* has a sulfatase enzyme to divert forming thiocyanates (Ratzka *et al.*, 2002) that could be interpreted as a way on which the folivore could improve its performance under more toxic conditions. Researchers have shown that insect weight is often positively correlated with foliar nitrogen concentrations (e.g., see Fajer, 1989). None of 2 insect performance correlates including population size and dry mass that were measured in the current study were affected by the presence or absence of other insect species. As expected, nitrogen content in both insect herbivores decreased in the mixed plantings. However, both fitness correlates of the phloem feeder insect did not show disparity under different treatments.

In contrast to specialist defensive compound, the pattern of plant allocation to common defensive compound (TP) as an index of antibiosis resistance was different under different insect phytophagy modes (phloem feeding vs. folivory). Besides, different insect phytophagy modes showed significant difference in inducing plant tolerance, which is known as a common plant response against different stresses. Against our expectation and according to the plant species responses, we conclude that phloem feeders are capable of inflicting more damage to plants than the folivores. So, it could be concluded that plant sap is more critical than leaves against insect herbivores and accordingly we conclude that phloem feeder is more harmful enemy to the plants than the folivore. As it was expected, N percentage decreased in both plant species after herbivory that could induce the plants to defend and compensate. Even though the plants' both general and special defenses decreased under interspecific interference, it could be suggested that specialist insect herbivores were more challenged in simpler systems that show higher defense responses (such as TP and  $\text{SCN}^-$ ) than in more complex systems (such as polycultures). In practice and considering tolerance and antibiotic response of the experimental crops under different insect guild's action, our results could have implications for integrated crop and pest management programs. And with caution, it could be suggested that managing phloem feeder insects is more effective than treating plants against folivores.

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## Disclosure

The authors declare that they have no conflict of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Analysis from the carbon, nitrogen and C : N ratio of the roots of the 2 experimental cultivars, *Brassica napus* and *Brassica juncea* under effect of plant species, planting set up, and insect phytophagy mode using a linear model approach.

**Table S2.** Correlation analysis of the defensive plant metabolites including TP and thiocyanate ( $\text{SCN}^-$ ) with population size and biomass of the folivore insect herbivore, *Plutella xylostella* and the phloem feeder insect

herbivore, *Lipaphis erysimi* under 3 plant assemblages and 2 different insect assemblages (single herbivore vs. competing herbivore) at late season under greenhouse condition.